REMARKS/ARGUMENTS

I. Status of the claims

Claims 1-2, 6-7, 11, 16-17, 21, 26, 31-33, 35, 39-41, 45, 46, 50, and 51 are amended and claims 82-99 are canceled. Claims 1-3, 6-7, 11-13, 16-17, 21-23, 26, 31-35, 39-41, 45-46, and 50-51 are pending with entry of this amendment.

II. Obviousness rejection

Claims 1-3, 6-7, 11-13, 16-17, 21-23, 26, 31-35, 39-41, 45-46, and 50-51 were rejected under 35 USC § 103 as allegedly obvious in view of any of Brandis I, II, or III (referred to herein as "Brandis") in view of Baker as evidenced by Cormier. The Office Action acknowledged that Brandis did not teach actually making or testing the E681R mutation in *Taq* polymerase (see, e.g., Office Action, page 3, last paragraph), but argued it would have been obvious to make such a mutation because Brandis taught that basic amino acids K and H possessed reduced discrimination and provided a motivation to make the E681R mutation because R is allegedly "specifically enumerated in the disclosure of Brandis..." (Office Action, top of page 4) and "for the purpose of providing a number of mutant polymerases with reduced discrimination against incorporation of a fluorescein dye..." (Office Action, bottom of page 4). The Office Action also argued that Baker taught that "...conservative amino acid substitutions can be made in protein sequences without affecting the function of the protein" (Office Action, middle of page 4). The Office Action further argued that one of skill would have had a reasonable expectation of success because Brandis tests 16 of 19 possible mutants, including other amino acids in the same group as R (Office Action, middle of page 4).

In response to Applicants' arguments that Brandis taught away from use of R and that R produced unexpectedly good results, the Office Action argued that one of ordinary skill in the art would have expected to find that different amino acid substitutions have superior discrimination depending on what type of labeled nucleotide was used in the particular assay involved in the determination. Specifically, the Office Action argued that "the ordinary artisan would have expected the exact levels of discrimination to differ based on the base, dye or linker

used in the assay" (Office Action, sentence spanning pages 11-12). In support of this statement, the Office Action cited Brandis (col. 6, line 27-37) as stating that, "The precise degree of discrimination will also vary in accordance with the specific fluorescently labeled nucleotide assayed, e.g., variations in base, dye, or linker. Mutant DNA polymerase of the invention may exhibit anywhere from a slight reduction in discrimination ... to complete elimination of discrimination." The Office Action argued that "the type of nucleotide (dCTP vs ddCTP) as well as the label (Tet(II) vs HEX-2-PA used in Brandis and Dr. Gelfand's declaration are different" (Office Action, page 11).

Applicants respectfully disagree. Applicants contend that the effect of the R mutation was in fact unexpectedly good and surprising and therefore deserving of a patent.

Recently, the U.S. Supreme Court affirmed the holding of *Graham* regarding obviousness. *See, KSR Int'l Co. v. Teleflex Inc.*, 127 S.Ct. 1727 (2007). Further, the Court affirmed that a combination of known elements that "more than yield a predicable result" or that is taught away from in the art is not obvious. *See, KSR*, 127 S.Ct. at 1731, citing with approval *United States v. Adams*, 383 U.S. 39 (1966). MPEP § 2145 (VII) summarizes some of the case law acknowledging that unexpectedly advantageous or superior properties are a basis for finding nonobviousness:

> A *prima facie* case of obviousness based on structural similarity is rebuttable by proof that the claimed compounds possess unexpectedly advantageous or superior properties. *In re Papesch*, 315 F.2d 381, 137 USPQ 43 (CCPA 1963) (Affidavit evidence which showed that claimed triethylated compounds possessed anti-inflammatory activity whereas prior art trimethylated compounds did not was sufficient to overcome obviousness rejection based on the homologous relationship between the prior art and claimed compounds.); *In re Wiechert*, 370 F.2d 927, 152 USPQ 247 (CCPA 1967) (a 7-fold improvement of activity over the prior art held sufficient to rebut *prima facie* obviousness based on close structural similarity).

The Federal Circuit has stated that evidence of unexpected results "may include data showing that a compound is unexpectedly superior in a property it shares with prior art compounds" and has held that "[e]vidence that a compound is unexpectedly superior in one of a spectrum of

common properties ... can be enough to rebut a *prima facie* case of obviousness." *See, In re Chupp*, 2 USPQ2d 1437 (Fed. Cir. 1987).

Applicants submit that:

(1) If one assumes the cited portion of Baker is accurate, i.e., that "conservative amino acid substitutions can be made in protein sequences without affecting the function of the protein," and one assumes, as the Office Action argues based on Cormier, that R is a conservative substitution relative to K and H, then the results of Gelfand (set forth in the Declaration of David Gelfand, filed January 3, 2003, hereinafter referred to as the "Gelfand Declaration") were unexpected because R was notably superior than K or H (more than 10-fold and 36-fold better, respectively) and thus affected the function of the protein to an unexpectedly greater extent than K or H;

(2) One of ordinary skill in the art would not have expected dramatic changes in ranking of amino acid substitutions between the assays used by Brandis and Gelfand because the labeled nucleotides used in the two assays, while not identical, were not *very* different.

The Baker quotation indicates the data from Gelfand was surprising

Regarding point (1) above, the rejection clearly relies on the quotation from Baker as evidence for reasonable expectation of success (e.g., "The ordinary artisan would have had a reasonable expectation of success that the particular R and Q mutants would have possessed reduced discrimination given that ... other amino acids in the same group as Q and R, respectively, possessed this property ***in view of the teachings of Baker***" (Office Action, page 4, emphasis added)). Therefore, it appears to be the Office Action's position that one of ordinary skill in the art would expect that substitutions with K, H, or R would result in roughly the same protein activity. However, the record is clear that R results in greatly better (reduced) discrimination than either H or K. *See*, Gelfand Declaration, Figure 1 and Schoenbrunner Declaration, paragraph 5. As shown in the table of paragraph 5 of the Schoenbrunner Declaration, R was 10.25 and 36.25 times better than K and H, respectively. Notably, this

improvement is better than the 7-fold improvement found to be unexpected in the *In re Wiechert* case cited above. Moreover, the results using the R mutation were best of all possible amino acid substitutions. Further, while R has a value of 0.04 in Figure 1 of the Gelfand assay, the next seven best substitutions range from 0.38-0.79. Thus, it appears that many of the better substitutions all hit a ceiling that is at least 10-fold worse than R. Accordingly, the Gelfand Declaration provides data showing the R produced surprisingly good results, especially when one takes the quotation from Baker into account.

Moreover, in view of the Brandis data, which shows that an M substitution as the best of the options tested, and H and K being seventh and eighth best, respectively (Brandis '193 patent, Col.8:9-15), it is particularly surprising that R was the best, as shown in the Gelfand Declaration. Indeed, this is supported by the Schoenbrunner Declaration, paragraph 8, which states:

> if R, H, and K reflected conservative amino acid changes, and one expected similar results for these substitutions, one would not have expected that R would show a 10-30-fold improvement relative to K or H (as observed by Gelfand) and one would certainly not have expected that the *ranking* of substitutions would change to such a great degree that R would be nearly 20-fold better than M. Indeed, if one of ordinary skill in the art expected conservative amino acid changes to have approximately the same effect, and then read the Brandis data, one would have been surprised to find that R was far superior than M as a substitution.

One of ordinary skill would not have expected a dramatic change in ranking of substitutions between the Brandis and Gelfand assays

As explained in the accompanying Schoenbrunner Declaration, the labeled nucleotides used in the Gelfand and Brandis assays were not *very* different. *See*, Schoenbrunner Declaration, paragraph 10. The difference in structure at the 3' portion of the nucleotides (dCTP used by Gelfand and ddCTP used by Brandis) is at the far end of the molecule relative to the label and thus polymerase amino acids that interact with the 3' portion would not be expected to affect discrimination against incorporation of fluorescein-labeled nucleotides. This is

summarized in paragraph 10 of the Schoenbrunner Declaration, which concludes that: "one of skill in the art would not expect that presence or absence of a 3' hydroxyl (the difference between the nucleotides in Brandis and Gelfand) would affect discrimination against incorporation of fluorescein-labeled nucleotides."

In addition, the difference between the labels in the labeled nucleotides used by Gelfand and Brandis were "relatively similar." *See*, Schoenbrunner Declaration, paragraph 11 and Figure accompanying Schoenbrunner Declaration. As explained in the Schoenbrunner Declaration, the difference between the two labels was only the presence or absence of two chloro moieties. Thus, the Schoenbrunner Declaration states:

> While one would not expect to be able to directly compare quantities between two different assays measuring discrimination, one would expect that the *ranking* of amino acid substitutions would be similar when the assays use similar labeled nucleotides as is the case here. For example, if one assay showed that M was best and that H and K provided middle-of-the-pack results, one of ordinary skill in the art would generally expect a second assay for measuring similarly-labeled nucleotide discrimination to yield essentially the same ranking of substitutions, i.e., M better than H or K. In view of the similarity between the HEX and TET labels, I would not have expected the relative discrimination between HEX and TET-labeled nucleotides to be significantly different between different amino acid substitutions at position 681. Therefore, it is my opinion that one of ordinary skill in the art would have been surprised to learn that substitution with R results in such a superior level of reduced discrimination compared to other basic amino acid substitutions such as H and K and results in the best substitution overall, having a nearly 20-fold improvement over M.

The Office Action had argued that that one of ordinary skill, reading Brandis (particularly col. 6:27-37), would have expected the exact *levels* of discrimination to differ based on base, dye, or linker used in the assay." *See*, Office Action, paragraph spanning pages 11-12 (emphasis added). As an initial matter, while *levels* may change, it is not clear that relative rankings would change.

Moreover, reference to the section of Brandis quoted in the Office Action, *but including some additional sentences from Brandis just before that section*, makes it clear that Brandis referred to *very significant* possible variation in nucleotides labels, including non-

fluorescein labels and even non-fluorescent labels that are not reflected in the differences between the Brandis and Gelfand assays.

> In addition to reduced discrimination against nucleotides labeled with fluorescein-type dyes, the mutant DNA polymerases of the invention may also exhibit reduced discrimination against ***nucleotides labeled with other fluorescent dyes that are not fluorescein-type dyes***, as well as reduced discrimination ***against other detectable moieties***. The fluorescently labeled nucleotides for which a given embodiment of the mutant DNA polymerases of the invention exhibit reduced discrimination may vary with respect to the particular fluorescent label, the linker used to attach the fluorescent label to the nucleotide, the site of attachment for the linker on the fluorescent label, the specific nucleotide base that is selected, and the site of attachment for the linker on the nucleotide. The precise degree of reduction in discrimination against a fluorescently labeled nucleotide will vary in accordance with the specific mutation or mutations introduced into the DNA polymerase. The precise degree of reduction in discrimination will also vary in accordance with the specific fluorescently labeled nucleotide assayed, e.g., variations in base, dye, or linker. Mutant DNA polymerase of the invention may exhibit anywhere from a slight reduction in discrimination against fluorescently labeled nucleotides to a complete elimination in discrimination, i.e., the mutant enzyme does not significantly differ with respect of rate of incorporation of labeled or unlabeled nucleotides.
>
> *See*, Brandis, '193 patent, Col.6:10-35 (emphasis added)

While such a huge difference in labeled nucleotide structure, such as comparing fluorescein with non-fluorescein labels, may result in some change in ranking of amino acid substitutions, the quotation taken in context does not imply that the smallest change would lead one of ordinary skill to expect significant rank changes for amino acid substitutions. Thus, Applicant contend that the data presented in the Gelfand Declaration (i.e., the activity of the R substitution) represents unexpected results.

Moreover, even if one of ordinary skill in the art would have expected variation between the Gelfand and Brandis assays (which Applicants dispute), there would have been no reasonable predictability in which amino acid would be best. The Gelfand Declaration indicates that R is best in the assay presented. This showing is sufficient under Federal Circuit case law. For example, the Federal Circuit stated the following in *In Re Chupp*, 2 USPQ2d 1437, 1439

(Fed. Cir. 1987) (disagreeing with the Solicitor regarding *In re Papesch*, 315 F.2d 381 (CCPA 1963)):

> We do not agree with the Solicitor's construction of *Papesch*. *Papesch* held that a compound can be patented on the basis of its properties; it did not hold that those properties must produce superior results in every environment in which the compound may be used. To be patentable, a compound need not excel over prior art compounds in all common properties. See *United States v. Ciba-Geigy Corp*., 508 F.Supp. 1157, 1169, 211 USPQ 529, 535-36 (D.N.J. 1979). Evidence that a compound is unexpectedly superior in one of a spectrum of common properties, as here, can be enough to rebut a prima facie case of obviousness. *In re Ackermann*, 444 F.2d 1172, 1176, 170 USPQ 340, 343 (CCPA 1971).

Here, like in *Chupp*, Applicants have provided data that show that a particular compound (a polymerase with the R substitution) produces unexpectedly superior results in a particular property, i.e., the assay presented in the Gelfand Declaration. Therefore, Applicants have provided sufficient data to rebut a *prima facie* obviousness rejection, to the extent such a rejection has been set forth.

It is not clear why one of ordinary skill would have made the R substitution if one would have expected no better results than Brandis saw for K or H

Finally, in view of the expectations set forth in Baker, it is not clear why one of ordinary skill would have been motivated to make the R substitution. According to the Office Action, one would have been motivated to make the E681R mutation because

(1) R is allegedly "specifically enumerated in the disclosure of Brandis..." (Office Action, top of page 4) and

(2)"for the purpose of providing a number of mutant polymerases with reduced discrimination against incorporation of a fluorescein dye..." (Office Action, bottom of page 4).

If one assumes that conservative amino acid substitutions result in approximately the same protein activity (per Baker), why would one make the R mutation when it was less preferable than other substitutions? The Brandis US Patent No. 6,265,193 states that possible substitutions "listed in the order of decreasing preference" are as follows: "M>I>W>L>V>P>H=K...." (Brandis '193 patent, col. 8:9-15). Consistent with the Baker

quotation, Brandis indicates H and K has the same activity ("...H=K..."). However, H and K are the seventh and eighth mutation of the list. Why would one of ordinary skill in the art make yet another mutation that has the seventh or eighth best activity when one could make an enzyme with the best activity (i.e., M, based on the Brandis data)? What does the Office Action mean in stating there was a "purpose of providing a number of variant polymerases...?" Isn't only one mutant polymerase needed at one time? Since Brandis did not make the R mutation, and indicated that H and K were barely in the top 50% of possible substitutions, Applicants contend that there was no motivation to make the R mutation.

Conclusion

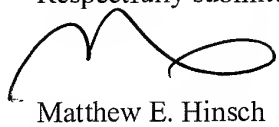
In view of the arguments presented above, Applicants respectfully request withdrawal of the pending obviousness rejections.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,



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